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Short Communication

Pathological and molecular investigation of velogenic neurotropic Newcastle disease outbreak in an organized Emu (*Dromaius novaehollandiae*) farm

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Abstract

Newcastle disease virus (NDV) poses a significant threat to avian species, including emus. This study reports an outbreak of velogenic NDV in an organised emu farm located in Andhra Pradesh characterized by high morbidity and mortality. Virus isolation was achieved during the first passage in embryonated chicken eggs, and molecular confirmation was performed via RT-PCR targeting the fusion protein cleavage site. Histopathological examination revealed systemic lesions, including encephalitis, pulmonary edema with pneumonia, proventricular epithelial desquamation, intestinal mucosal necrosis, renal hemorrhage and necrosis. The outbreak highlighted the inefficacy of the currently used vaccine strains, underscoring the urgent need for genotype-matched vaccines to enhance protection. These findings provide critical insights into the pathology and molecular characteristics of velogenic NDV, emphasizing improved diagnostic and preventive strategies for managing NDV in emus and other avian species.

Keywords: Emu, Histopathology, Newcastle disease, PCR, Velogenic NDV

Highlights

- Report of a velogenic NDV outbreak causing high mortality in an Indian emu farm.
- NDV isolated in embryonated chicken eggs showing typical pathological changes in embryos.
- Molecular confirmation of NDV by RT-PCR targeting the fusion protein cleavage site (FPCS).
- Insights into NDV pathology, molecular diagnostics, and control strategies in exotic poultry.

Poultry farming is one of the most dynamic and rapidly evolving segments in the Indian agricultural sector. Concomitantly, poultry are highly vulnerable to several infectious diseases, leading to production losses, thus, a negative impact on the nation's economy. Vaccination regimes are well established in commercial chicken, while, other commercial poultry, including ducks, emus, turkeys and migratory/wild birds suffer lack of proper disease surveillance and control systems.

Newcastle disease caused by Avian Orthoavulavirus-1 (AOAV-1), or NDV, affects more than 240 avian species all over the world. Based on the severity of the disease, NDV strains are classified into three categories: lentogenic (apathogenic), mesogenic (intermediate pathogenic), and velogenic (highly pathogenic). Velogenic NDV causes serious outbreaks resulting in massive mortality in the affected birds, while lentogenic NDV is non-pathogenic and birds like migratory/wild waterfowls act as reservoirs. Several ND outbreaks in various avian species have been reported previously, in commercial and backyard chicken (Gowthaman et al., 2016, 2018), emus (Gowthaman et al., 2016, Deepthi et al., 2021), peacock (Khulape et al., 2014), pigeons (Tirumurugaan et al., 2011, Deepthi et al., 2024), quails (Bhuvaneswari et al., 2014), pelicans (Deepthi et al., 2022) and ducks (Roy et al., 2000). The present study reports detection and characterization of NDV in an organised emu farm housing a total of 350 birds including 12-year old parent stock (n= 200) and 9-month old chicks (n=150) in Nunna, Andhra Pradesh, India, where sudden heavy mortality was recorded during October and November, 2024. Clinical signs first appeared in emu birds on October 6, 2024, initially affecting young individuals with severe respiratory discharge, depression, and inappetence, leading to mortality in a few juveniles. Adults began showing symptoms approximately one week later, with a few adult birds also exhibiting mortality, and samples were collected on the 12th day of the outbreak. The chicks received an NDV-F1 vaccine at four weeks of age.

Clinical symptoms appeared first in the chicks and subsequently spread to the adult breeding flock. The outbreak resulted in high morbidity and mortality, with approximately 90% of the chicks affected, and about 60% mortality among the parent stock. This contrasts

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sharply with reports by Gowthaman et al. (2016), who documented only 0.4–2.5% mortality in emu flocks located in poultry-dense regions of India such as Hyderabad, Namakkal, and Bareilly. Despite emus being susceptible to Newcastle disease virus (NDV), their relatively low mortality rates may be attributed to a certain degree of innate or indigenous resistance to the virus. Supporting this, Deepthi et al. (2021) reported isolation of velogenic NDV from apparently healthy emu flocks, suggesting subclinical infections or tolerance within these populations. These findings highlight species-specific differences in NDV susceptibility and underscore the importance of considering host factors and resistance mechanisms when evaluating disease impact in various bird species.

Early clinical findings in sick birds included loss of appetite, lethargy, collapse, and later, greenish diarrhea. Severe neurological signs such as circling, leg paddling, and paralysis developed shortly before death (Fig. 1). Post-mortem examinations of the deceased birds revealed notable gross lesions, including congestion in the brain, liver, kidneys, trachea, and intestines; lung consolidation; blackish discoloration of the caecal tonsils; and catarrhal contents with severe mucosal congestion in the intestinal lumen (Fig. 2).

From each bird necropsied, pooled tissue samples comprising spleen, lung, liver, trachea, and intestines were collected, along with individual brain samples. These tissues were prepared as 10% (w/v) homogenates and subjected to virus isolation through inoculation into embryonated chicken eggs, following the standard procedure described in the World Organisation for Animal Health [OIE] (2012). Presence of NDV was confirmed by hemagglutination, hemagglutination inhibition tests, and RT-PCR. The Lasota vaccine strain from IVPM, Ranipet, India, served as antigen and positive control. The virus was isolated successfully during the first passage, and the embryos that were harvested showed typical pathological signs such as hemorrhages near the sub-occipital region. The allantoic fluid obtained from these embryos exhibited a hemagglutination titer of 1:512 per 50 microliters. Additionally, the hemagglutination inhibition test, performed using polyclonal chicken antiserum specific to the LaSota strain, yielded a titer of 1:256, confirming the isolate as Avian Orthoavulavirus-1 (AOAV-1).

The virus was successfully isolated during the first passage in embryonated chicken eggs, a well-established and widely used method for Newcastle disease virus (NDV) isolation (Alexander & Senne, 2008; World Organisation for Animal Health [WOAH], 2021). The harvested embryos exhibited typical pathological lesions such as hemorrhages near the sub-occipital region, which are characteristically associated with NDV infection (King, 1996). The allantoic fluid from these embryos showed a high hemagglutination titer of 1:512 per 50 microliters, indicating active viral replication (Miller & Koch, 2013). Further confirmation was achieved through hemagglutination inhibition (HI) testing using polyclonal chicken antiserum specific to the LaSota strain, which yielded a titer of 1:256, thereby validating the isolate as Avian Orthoavulavirus-1 (AOAV-1) (Miller et al., 2010; WOAH, 2021). These methods remain the gold standard for NDV diagnosis due to their sensitivity and specificity (Lamb et al., 2020). The combined use of virus isolation, hemagglutination, and HI assays not only confirms the presence of the virus but also ensures accurate identification, essential for epidemiological studies and vaccine quality control.

Molecular confirmation of Newcastle Disease Virus (NDV) in this study was accomplished through reverse transcription PCR (RT-PCR), a highly sensitive and specific diagnostic tool widely adopted (Emeru et al., 2024; Siddique & Ali, 2025). RNA extraction from the amnioallantoic fluid using TRIzol reagent followed by cDNA synthesis with random primers aligns with established molecular protocols for NDV detection (Siddique & Ali, 2025). PCR targeting the fusion protein cleavage site (FPCS) of NDV, a critical determinant of virulence, was performed using primers [Forward: (5'-ACG AGA GAG GAT GAT GAT GAG GTC AGC G-3') and Reverse: (5'-GCA GAG GTT GAT GAT GAT GAC GCA GTT C-3')] described by Nanthakumar et al. (2000). PCR was performed with conditions including initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45s, 60°C for 1 min, and 72°C for 1 min, a final extension at 72°C for 10 min, and hold at 4°C. This conserved genetic region of the fusion gene (356 bp fragment) is routinely used for differentiation and molecular characterization of NDV strains (Alemu et al., 2024; Siddique & Ali, 2025). Recent studies underscore the advantage of RT-PCR in rapid and accurate NDV detection over conventional assays, enabling effective surveillance, outbreak control, and vaccine efficacy evaluation (Emeru et al., 2024; Berihulay et al., 2025). Thus, the use of RT-PCR targeting FPCS ensures reliable confirmation of AOAV-1 presence in clinical samples, complementing classical virological tests.

Additionally, histopathological evaluation was performed on the tissues collected. Sections of 4-5 μm thickness were prepared from various tissues and

stained with haematoxylin and eosin (H&E) as per the conventional method (Culling, 1968). The predominant microscopic lesions were observed in the brain, where changes were consistent with encephalitis, marked by perivascular lymphocytic cuffing, neuronal degeneration, neuronophagia, chromatolysis, and satellitosis, paralleling observations made by Nooruzzaman et al. (2022) in chickens infected with a velogenic NDV strain. In the lungs, alterations included pronounced pulmonary edema, severe congestion, emphysematous changes, thickening of alveolar septa, and fibrinous pneumonia, aligning with documented respiratory pathology indicating primary viral replication in pulmonary tissues (Fig. 3). The proventriculus exhibited desquamation of the glandular epithelium along with central glandular hemorrhages, similar to lesions reported by Wakenell et al. (2021), indicative of direct viral cytopathic effects and secondary inflammatory responses. Intestinal tissues revealed widespread mucosal hemorrhages and necrosis. Renal lesions were characterized by extensive hemorrhage, congestion, and necrosis of the tubular epithelium.

The outbreak of velogenic Newcastle Disease Virus (NDV) in the emu farm resulted in severe clinical disease, high morbidity, and mortality, reflecting the aggressive nature of velogenic strains in susceptible avian species. Compared to previous reports indicating low mortality in emu flocks, this outbreak underscores the potential for severe disease under certain conditions, possibly influenced by viral virulence, host susceptibility, and various ecological factors such as environmental stressors (e.g., high ambient temperatures, humidity fluctuations, poor ventilation, or overcrowding in enclosures) that enhance viral stability and transmission, as well as interspecies transmission risks including exposure to wild birds (e.g., pigeons, cormorants, waterfowl, or other resident species acting as reservoirs). This emphasizes the necessity of stringent biosecurity measures, continuous surveillance, and timely vaccination protocols to prevent and control NDV outbreaks in emu farms and other poultry species. This study also highlights that currently used vaccine strains (often genotype II) are becoming less effective in controlling Newcastle disease due to antigenic drift and genotype mismatches with emerging strains like genotype VII, emphasizing the urgent need to develop and implement genotype-matched vaccines for improved protection and disease management. The comprehensive virological and pathological confirmation in this study provides valuable insights for managing NDV in exotic and domesticated birds alike.

Conflict of interest: The authors declare that they have no conflict of interest.

Author's contribution: BD: Conceptualized and designed the study as well as performed virus isolation and molecular assays; JJL: Conducted the field study and helped in the collection of samples. Both authors contributed to data interpretation, drafted and reviewed the manuscript critically, and approved the final version for publication.

Data availability statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval: Ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) of NTR College of Veterinary Science, Gannavaram, under approval number 5/IAEC/NTRCVSC/2018. All efforts were made to minimize animal suffering during sample collection and experimentation.

Use of artificial intelligence tools: During the preparation of the work, the authors used Grammarly to check proper use of grammar and spell check. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Fig. 1. Affected emu showing nervous signs like circling, paddling and paralysis of legs

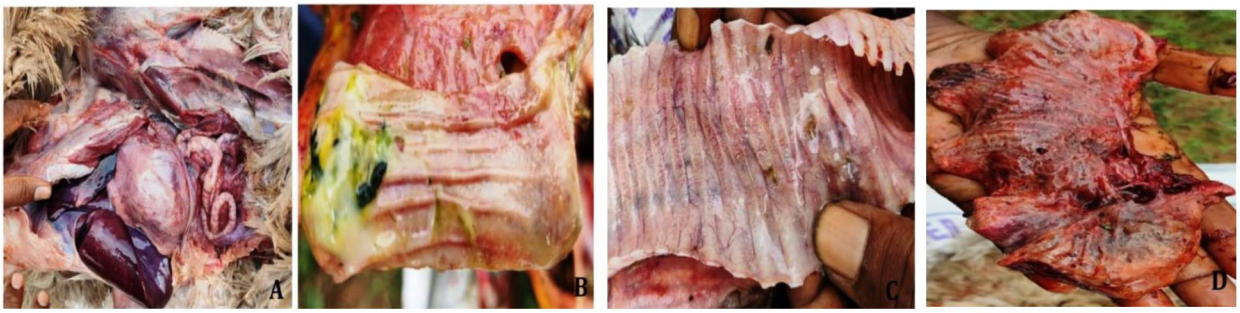




Fig. 2. Gross lesions observed in the ND affected emus. A. Congestion in the viscera; B. Catarrhal contents with severe mucosal congestion in the intestinal lumen; C. Haemorrhages in trachea; D. Haemorrhages and consolidation in lungs; E. Blackish discolouration in the crop

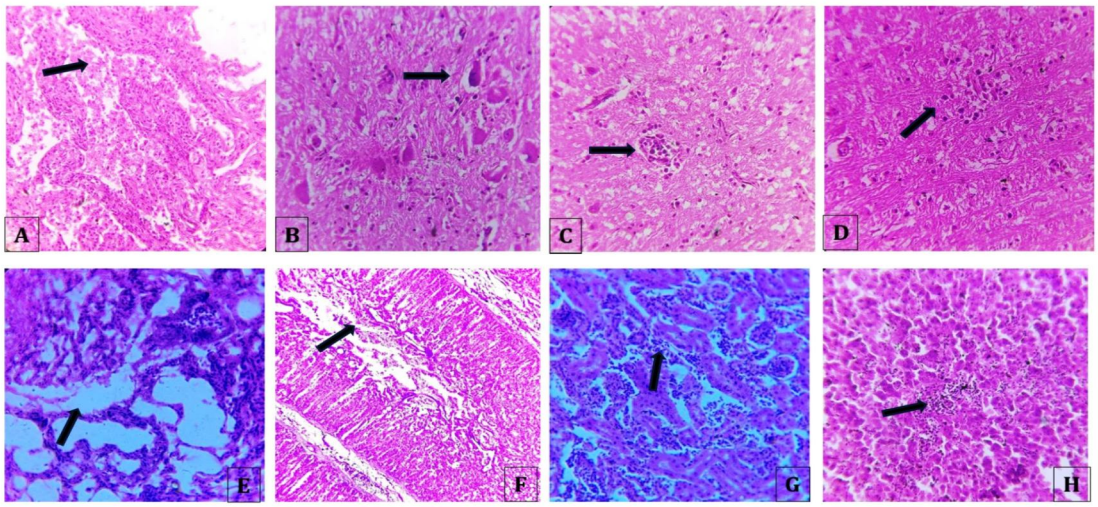


Fig. 3. H & E stained tissue sections from deceased emu birds. A. Haemorrhages in meninges; B. Chromatolysis and satellitosis in neurons; C. Perivascular cuffing of lymphocytes in brain; D. Neuronophagia; E. Lungs- Emphysema and thickening of intra alveolar septa; F. Proventriculus- Central glandular haemorrhages; G. Kidneys- Severe haemorrhage and necrosis of tubular epithelium; H. Liver- Sinusoidal congestion and individualization of hepatocytes

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